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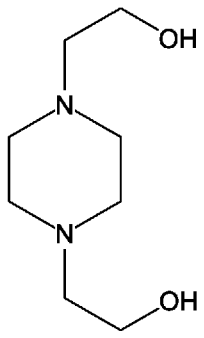

FIG. 6

(57) Abstract: Embodiments of the disclosed invention provide devices that mitigate salinity-based EAB sensor output variability by improving aptamer structural rigidity or sensing performance through the pretreatment of aptamers with ions or buffering molecules. Other embodiments provide devices that mitigate salinity- based EAB sensor output variability through the pretreatment of additional device components with ions or buffering molecules thereby reducing ion uptake from, or output to, a biofluid sample.

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SALINITY-STABILIZED EAB BIOSENSORS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/618,778, filed January 18, 2018, and has specification that relates to PCT/US16/58357, filed October 23, 2016, and PCT/US17/23399, filed March 21, 2017, the disclosures of which are hereby incorporated by reference herein in their entirety.

BACKGROUND OF THE INVENTION

[0002] Wearable biosensing technologies have enormous potential for several medical, fitness, and personal well-being applications. Sweat contains many of the same biomarkers, chemicals, or solutes that are carried in blood and interstitial fluid, and can provide significant information enabling one to diagnose illness, health status, exposure to toxins, performance, and other physiological attributes even in advance of any physical sign. Interstitial fluid has even more analytes nearer to blood concentrations than sweat does, especially for larger sized and more hydrophilic analytes (such as proteins). Sweat itself, the action of sweating, and other parameters, attributes, solutes, or features on, near, or beneath the skin can be measured to further reveal physiological information. However, recent progress in the development of wearable sweat sensing devices has been limited to high concentration analytes (μM to mM) sampled at high sweat rates (>1 nL/min/gland) found in, for example athletic applications. Progress will be much more challenging as wearable biosensing moves towards detection of large, low concentration analytes (nM to pM and lower).

[0003] In particular, many known sensor technologies for detecting larger molecules are ill-suited for use in wearable biofluid sensing, which requires sensors that permit continuous or extended use on a wearer's skin. This means that sensor modalities that require complex microfluidic manipulation, the addition of reagents, or the use of limited shelf-life components, such as antibodies, will not be sufficient for such sensing. What is needed is a stable, reliable, reagentless sensor that is sensitive to target analytes in biofluid, while providing the level of specificity necessary to produce high predictive values during the lifespan of the sensor. One solution to this problem is the use of electrochemical aptamer-based ("EAB") sensor technology, such as the multiple-capture EAB biosensors ("MCAS") disclosed in U.S. Patent Nos.

7,803,542 and 8,003,374, or the docked aptamer EAB biosensors ("DAS") disclosed in U.S. Provisional Application No. 62/523,835, filed June 23, 2017, each of which is hereby incorporated by reference herein in its entirety.

[0004] However, one challenge with the use of EAB sensor technology in biofluid is that electrical outputs from such sensors often have a strong dependence on biofluid sample salinity or ion content, and sweat salinity in particular can vary widely. The salinity dependence seen in EAB sensors is primarily due to the effect salinity has on the structural rigidity of aptamers and other components of an analyte capture complex, which influences the strength and valence of electrical signals indicating analyte capture. As a consequence, salinity variability can have a significant effect on an EAB sensor's response to a target analyte.

[0005] Aptamers display such salinity-based structural variance due to their inherent structural flexibility. Aptamer sequences are lengthy chains of nucleotides having extensive freedom of movement, including the ability to fold into secondary and sometimes tertiary structures. This characteristic makes the behavior of aptamers difficult to predict, especially when the physical configurations are exposed to wide variations in biofluid sample salinity or ion content. One solution would be to add ion sensors, and use the ion concentration measurements to correct for salinity-induced errors. For example, integration of ion selective electrode sensors for Na^+, Cl^-, K^+, and NH_4^+ into a sweat biosensing device is disclosed in PCT/US15/40113. Another solution would be to buffer the biofluid sample for salinity, as disclosed in PCT/US16/58357. However, for many applications, adding sensors to measure and compensate for salinity, or adding components to buffer the biofluid sample may prove inferior to reducing aptamer salinity variability.

[0006] Therefore, what is needed are simple, yet robust devices and methods to reduce the output variability due to salinity for EAB sensors in a biofluid sensing device. Disclosed herein are aptamer configurations that display improved structural rigidity due to pretreatment with ions or buffering molecules.

SUMMARY OF THE INVENTION

[0007] Embodiments of the disclosed invention provide devices that mitigate salinity-based EAB sensor output variability by improving aptamer structural rigidity or sensing performance through the

pretreatment of aptamers with ions or buffering molecules. Other embodiments provide devices that mitigate salinity-based EAB sensor output variability through the pretreatment of additional device components with ions or buffering molecules thereby reducing ion uptake from, or output to, a biofluid sample.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] The objects and advantages of the disclosed invention will be further appreciated in light of the following detailed descriptions and drawings in which:

[0009] Figs. 1A & 1B are representations of a previously disclosed MCAS EAB sensing element.

[0010] Figs. 2A & 2B are representations of a previously disclosed DAS EAB sensing element.

[0011] Fig. 3 is a depiction of a previously disclosed biofluid sensing device configured to use EAB sensors to measure one or more biofluid characteristics.

[0012] Fig. 4 is a depiction of the structure of a DNA molecule.

[0013] Figs. 5A & 5B depict buffering molecules for aptamer or other component pretreatment according to the disclosed invention.

[0014] Fig. 6 is a depiction of a buffering molecule for aptamer or other component pretreatment according to the disclosed invention.

[0015] Fig. 7 is a depiction of a buffering molecule for aptamer or other component pretreatment according to the disclosed invention.

[0016] Fig. 8 is a depiction of a buffering molecule for aptamer or other component pretreatment according to the disclosed invention.

[0017] Fig. 9 is a depiction of ion interaction with a cellulose molecule.

DEFINITIONS

[0018] Before continuing with a detailed description of the exemplary embodiments, a variety of definitions should be made, these definitions gaining further appreciation and scope in the detailed description and embodiments of the present disclosure.

[0019] As used herein, "sweat" or "sweat biofluid" means a biofluid that is primarily sweat, such as eccrine or apocrine sweat, and may also include mixtures of biofluids such as sweat and blood, or sweat

and interstitial fluid, so long as advective transport of the biofluid mixtures (*e.g.*, flow) is primarily driven by sweat.

[0020] As used herein, "biofluid" may mean any human biofluid, including, without limitation, sweat, interstitial fluid, blood, plasma, serum, tears, and saliva. A biofluid may be diluted with water or other solvents inside a device because the term biofluid refers to the state of the fluid as it emerges from the body.

[0021] As used herein, "interstitial fluid" is a solution that bathes and surrounds tissue cells. The interstitial fluid is found in the interstices between cells. Embodiments of the disclosed invention measure analytes from interstitial fluid found in the skin and, particularly, interstitial fluid found in the dermis. In some cases where interstitial fluid is emerging from sweat ducts, the interstitial fluid contains some sweat as well, or alternately, sweat may contain some interstitial fluid.

[0022] As used herein, "fluid" may mean any human biofluid, or other fluid, such as water, including without limitation, groundwater, sea water, freshwater, etc., or other fluids.

[0023] As used herein, "continuous monitoring" means the capability of a device to provide at least one sensing and measurement of fluid collected continuously or on multiple occasions, or to provide a plurality of fluid measurements over time.

[0024] "Biofluid sensor" means any type of sensor that measures a state, presence, flow rate, solute concentration, solute presence, in absolute, relative, trending, or other ways in a biofluid. Biofluid sensors can include, for example, potentiometric, amperometric, impedance, optical, mechanical, antibody, peptide, aptamer, or other means known by those skilled in the art of sensing or biosensing.

[0025] "Analyte" means a substance, molecule, ion, or other material that is measured by a biofluid sensing device.

[0026] "Measured" can imply an exact or precise quantitative measurement and can include broader meanings such as, for example, measuring a relative amount of change of something. Measured can also imply a binary or qualitative measurement, such as 'yes' or 'no' type measurements.

[0027] As used herein, "determined" may encompass more specific meanings including but not limited to: something that is predetermined before use of a device; something that is determined during use of a device; something that could be a combination of determinations made before and during use of a device.

[0028] As used herein, "chronological assurance" is an assurance of the sampling rate for measurement(s) of sweat, interstitial fluid (or other biofluid or fluid), or solutes in biofluid, being the rate at which measurements can be made of new biofluid or its new solutes as they originate from the body. Chronological assurance may also include a determination of the effect of sensor function, or potential contamination with previously generated biofluid, previously generated solutes, other fluid, or other measurement contamination sources for the measurement(s).

[0029] "EAB sensor" means an electrochemical aptamer-based biosensor that is configured with multiple aptamer sensing elements that, in the presence of a target analyte in a fluid sample, produce a signal indicating analyte capture, and which signal can be added to the signals of other such sensing elements, so that a signal threshold may be reached that indicates the presence or concentration of the target analyte. Such sensors can be in the forms disclosed in U.S. Patent Nos. 7,803,542 and 8,003,374 (the "Multi-capture Aptamer Sensor" (MCAS)), or in U.S. Provisional Application No. 62/523,835 (the "Docked Aptamer Sensor" (DAS)).

[0030] "Analyte capture complex" means an aptamer, or other suitable molecules or complexes, such as proteins, polymers, molecularly imprinted polymers, polypeptides, and glycans, that experience a conformation change in the presence of a target analyte, and are capable of being used in an EAB sensor. Such molecules or complexes can be modified by the addition of one or more linker sections comprised of nucleotide bases.

[0031] "Aptamer sensing element" means an analyte capture complex that is functionalized to operate in conjunction with an electrode to detect the presence of a target analyte. Such functionalization may include tagging the aptamer with a redox moiety, or attaching thiol binding molecules, docking structures, or other components to the aptamer. Multiple aptamer sensing elements functionalized on an electrode comprise an EAB sensor.

[0032] "Sensitivity" means the change in output of the sensor per unit change in the parameter being measured. The change may be constant over the range of the sensor (linear), or it may vary (nonlinear).

[0033] "Signal threshold" means the combined strength of signal-on indications produced by a plurality of aptamer sensing elements that indicates the presence of a target analyte.

[0034] As used herein, "sample generation rate" is the rate at which biofluid is generated by flow through pre-existing pathways. Sample generation rate is typically measured by the flow rate from each pre-existing pathway in nL/min/pathway. In some cases, to obtain total sample flow rate, the sample generation rate is multiplied by the number of pathways from which the sample is being sampled. Similarly, as used herein, "analyte generation rate" is the rate at which solutes move from the body or other sources toward the sensors.

[0035] As used herein, "biofluid sampling rate" or "sampling rate" is the effective rate at which new biofluid, originating from pre-existing pathways, reaches a sensor that measures a property of the fluid or its solutes. Sampling rate is the rate at which new biofluid is refreshed at the one or more sensors and therefore old biofluid is removed as new fluid arrives. In one embodiment, this can be estimated based on volume, flow-rate, and time calculations, although it is recognized that some biofluid or solute mixing can occur. Sampling rate directly determines or is a contributing factor in determining the chronological assurance. Times and rates are inversely proportional (rates having at least partial units of 1/seconds), therefore a short or small time required to refill sample volume can also be said to have a fast or high sampling rate. The inverse of sampling rate (1/s) could also be interpreted as a "sampling interval(s)". Sampling rates or intervals are not necessarily regular, discrete, periodic, discontinuous, or subject to other limitations. Like chronological assurance, sampling rate may also include a determination of the effect of potential contamination with previously generated biofluid, previously generated solutes (analytes), other fluid, or other measurement contamination sources for the measurement(s). Sampling rate can also be in part determined from solute generation, transport, advective transport of fluid, diffusion transport of solutes, or other factors that will impact the rate at which new sample will reach a sensor and/or is altered by older sample or solutes or other contamination sources.

[0036] As used herein, "sample generation rate" is the rate at which biofluid is generated by flow through pre-existing pathways. Sample generation rate is typically measured by the flow rate from each pre-existing pathway in nL/min/pathway. In some cases, to obtain total sample flow rate, the sample generation rate is multiplied by the number of pathways from which the sample is being sampled. Similarly, as used herein, "analyte generation rate" is the rate at which solutes move from the body or other sources toward the sensors.

DETAILED DESCRIPTION OF THE INVENTION

[0037] Experimental evidence indicates that the salinity of a sweat sample being measured, and the rigidity of the aptamer component within an EAB sensing element directly affect the sensitivity, selectivity, and recovery interval of EAB sensors. It is therefore desirable to minimize or control the EAB sensor performance variabilities caused by biofluid sample salinity variations through the pretreatment of aptamer sequences or other device components that come into contact with the biofluid sample.

[0038] One skilled in the art will recognize that the various embodiments may be practiced without one or more of the specific details described herein, or with other replacement and/or additional methods, materials, or components. In other instances, well-known structures, materials, or operations are not shown or described in detail herein to avoid obscuring aspects of various embodiments of the invention. Similarly, for purposes of explanation, specific numbers, materials, and configurations are set forth herein in order to provide a thorough understanding of the invention. Furthermore, it is understood that the various embodiments shown in the figures are illustrative representations and are not necessarily drawn to scale.

[0039] Reference throughout this specification to "one embodiment" or "an embodiment" means that a particular feature, structure, material, or characteristic described in connection with the embodiment is included in at least one embodiment of the invention, but does not denote that they are present in every embodiment. Thus, the appearances of the phrases "in an embodiment" or "in another embodiment" in various places throughout this specification are not necessarily referring to the same embodiment of the invention. Further, "a component" may be representative of one or more components and, thus, may be used herein to mean "at least one."

[0040] Certain embodiments of the invention show sensors as simple individual components. It is understood that many sensors require two or more electrodes, reference electrodes, or additional supporting technology or features that are not captured in the description herein. Sensors are preferably electrical in nature, but may also include optical, chemical, mechanical, or other known biosensing mechanisms. Sensors can be in duplicate, triplicate, or more, to provide improved data and readings. Sensors may be referred to by what the sensor is sensing, for example: a sweat sensor; an impedance sensor; a fluid volume sensor; a sweat generation rate sensor; and a solute generation rate sensor. Certain embodiments of the disclosed invention show sub-components of what would be fluid sensing devices with more sub-

components needed for use of the device in various applications, which are obvious (such as a battery), and for purpose of brevity and focus on inventive aspects are not explicitly shown in the diagrams or described in the embodiments of the invention. As a further example, many embodiments of the invention could benefit from mechanical or other means known to those skilled in wearable devices, patches, bandages, and other technologies or materials affixed to skin, to keep the devices or sub-components of the skin firmly affixed to skin or with pressure favoring constant contact with skin or conformal contact with even ridges or grooves in skin, and are included within the spirit of the disclosed invention. The present application has specification that builds upon PCT/US13/35092, the disclosure of which is hereby incorporated herein by reference in its entirety.

[0041] The detailed description of the present invention will be primarily, but not entirely, limited to devices, methods and sub-methods using wearable biofluid sensing devices. Therefore, although not described in detail here, other essential steps which are readily interpreted from or incorporated along with the present invention shall be included as part of the disclosed invention. The disclosure provides specific examples to portray inventive steps, but which will not necessarily cover all possible embodiments commonly known to those skilled in the art. For example, the specific invention will not necessarily include all obvious features needed for operation. The invention includes reference to the article in press for publication in the journal IEEE Transactions on Biomedical Engineering, titled "Adhesive RFID Sensor Patch for Monitoring of Sweat Electrolytes"; the article published in the journal *AIP Biomicrofluidics*, 9 031301 (2015), titled "The Microfluidics of the Eccrine Sweat Gland, Including Biomarker Partitioning, Transport, and Biosensing Implications"; as well as PCT/US16/36038, and U.S. Provisional Application No. 62/327,408, each of which is included herein by reference in their entirety.

[0042] The disclosed invention applies at least to any type of fluid sensor device that measures fluid, fluid generation rate, fluid chronological assurance, its solutes, solutes that transfer into fluid from skin, tissue, or other source, a property of or things on the surface of skin, or properties or things beneath the skin. The invention applies to fluid sensing devices which can take on forms including patches, bands, straps, portions of clothing, wearables, or any suitable mechanism that reliably brings sweat stimulating, fluid collecting, and/or fluid sensing technology into intimate proximity with biofluid as it is generated. Some embodiments of the invention utilize adhesives to hold the device near the skin, but devices could

also be held by other mechanisms that hold the device secure against the skin, such as a strap or embedding in a helmet.

[0043] With reference to Fig. 1A, a previously disclosed MCAS aptamer sensing element is depicted. While the figure depicts, and the discussion involves, a single aptamer sensing element, EAB sensors described herein will include a large number (thousands, millions, or billions of individual sensing elements, having an upper limit of $10^{14}/cm^2$) attached to the electrode. The aptamer sensing element 110 includes an analyte capture complex 112, which in turn is comprised of a randomized aptamer sequence 140 that is selected to interact with a target analyte 160, and one or more linker nucleotide sections 142 (one is depicted). The analyte capture complex 112 has a first end covalently bonded to a sulfur molecule, *e.g.*, a thiol 120, which is in turn covalently bonded to an electrode base 130. The electrode 130 may be comprised of gold or another suitable conductive material. The sensing element further includes a redox moiety 150 that may be covalently bonded to a second end of the analyte capture complex or bound to it by a linking section. In the absence of the target analyte, the aptamer 140 is in a first configuration, and the redox moiety is in a first position relative to the electrode. When the device interrogates the sensing element using, *e.g.*, square wave voltammetry, the sensing element produces a first electrical signal, eT_A.

[0044] With reference to Fig. 1B, when the aptamer 140 interacts with a target analyte 160, the aptamer undergoes a conformation change that partially disrupts the first configuration, and forms a second configuration. The capture of the target analyte accordingly moves the redox moiety 150 into a second position relative to the electrode 130. Now when the biofluid sensing device interrogates the sensing element, the sensing element produces a second electrical signal, eT_B that is distinguishable from the first electrical signal, eT_A. After a recovery interval, the aptamer releases the target analyte, and the aptamer will return to the first configuration, which will produce the corresponding first electrical signal when the sensing element is interrogated.

[0045] With reference to Fig. 2A, a previously disclosed DAS aptamer sensing element is depicted. The aptamer sensing element 210 includes an analyte capture complex 212 and a molecular docking structure 220 immobilized on an electrode 230. The docking structure may be attached to the electrode by covalently bonding a first end to a thiol, which is, in turn, covalently bonded to the electrode. The docking structure includes a nucleotide sequence that is selected to be complementary with a nucleotide sequence

on the analyte capture complex 212; in this depiction, the dock is configured to pair with a first primer section 242. A redox chemical moiety 250 is immobilized on the unattached end of the dock 220, on the opposite end of the dock from the electrode. The dock further includes two complementary nucleotide sequences 222, 224. In the initial arrangement, the analyte capture complex is attached to the dock that is in turn attached to the electrode. When the dock is bound to the analyte capture complex, it is stiffened so that the redox moiety is located at a maximum distance from the electrode, thereby producing a first signal prior to analyte capture, eT_A.

[0046] With reference to Fig. 2B, the DAS is exposed to a biofluid sample containing a concentration of the target analyte 260. Upon interaction with the target analyte, the aptamer 240 interacts with the analyte, causing the second primer 244b to move into physical proximity to the first primer 242b. The physical proximity of the complementary primers causes the first primer to break free from the dock 220 and bind to the second primer, allowing the complex to move away from the dock. Once the dock is unbound from the first primer 242b, the dock becomes more flexible, and the complementary sections 222b, 224b bond together. The folding of the dock caused by the bonding sections 222b, 224b locks the attached redox moiety 250 in a position closer to the electrode 230, thereby producing a second signal, eT_B, upon interrogation.

[0047] With reference to Fig. 3, a cross-sectional view of at least a portion of a previously-disclosed biofluid sensing device employing EAB sensors is depicted. The device 300 includes a water-impermeable substrate 310, a protective covering 312, a microfluidic channel 380, an inlet 382, and a sweat collector (not shown) to introduce a biofluid sample into the device. The channel 380 is configured to concentrate a biofluid sample relative to a target analyte, and includes an optional pre-concentration filter 392, a selectively-permeable concentrator membrane 390 and a concentrator pump 394. When a biofluid sample enters the channel through the inlet, it moves in the direction of the arrow 16, where it encounters the pre-filter. The filter removes solutes from the biofluid sample based on size, electrical charge, chemical property, or other property, or removes proteases or other solutes that may interfere with the device measurements. Once through the filter, the biofluid sample is concentrated relative to the target analyte by the concentrator membrane 390, which could be a dialysis membrane or other material that at least allows the passage of water and inorganic solutes, but prevents passage of the target analyte. The pump 394 is

constructed of a material suitable for drawing water out of the channel through the membrane, or may be an osmotic draw pump.

[0048] As the biofluid sample moves through the channel, it becomes increasingly concentrated relative to the target analyte, and interacts with at least one EAB sensor 322, 324 and one or more optional secondary sensors 321, 323. Said secondary sensors may be one of the following: a micro-thermal flow rate sensor, one or more ion-selective electrodes ("ISE") for measuring electrolytes (H^+, Na^+, Cl^-, K^+ Mg^{2+}, etc.), a conductivity sensor, a temperature sensor, or other sensor. Some embodiments also include optional sweat stimulation capability, comprising a sweat stimulant gel 340 composed of, for example, agar and a sweat stimulant such as carbachol or pilocarpine, and an iontophoresis electrode 350. The electrode 350 can also be used to measure skin impedance or galvanic skin response, which indicates sweat onset or sweat cessation timing.

[0049] As disclosed herein, one means of rendering EAB sensors more stable in salinity-variable biofluids such as sweat, is to pretreat the aptamer sequences with ions, or with certain small molecules. Small molecule treatment of DNA and RNA molecules is well-known in the art. For example, urea can build up on double-stranded DNA, causing the strands to decouple. This has been exploited to develop urea-based decoupling processes such as denaturing urea polyacrylamide gel electrophoresis. As another example, it is known by practitioners in the art that DNA and RNA become more rigid, *i.e.*, less susceptible to conformation changes, in highly saline environments. This is mainly because chelated cations, primarily sodium in sweat, alter existing electrical forces and sterically reinforce the nucleotide structure, both of which tend to render the nucleotide structure more rigid. These cations in solution interact with the nucleotide bases, negatively charged phosphate molecules, and purine heterocycles. By contrast, the folding dynamics and characteristics for most proteins are dominated by hydrophobic collapse, rather than chelation of cations, since negatively charged residues or side-chains that serve as cation binding sites on most proteins are generally not numerous enough or close enough to contribute significant steric rigidity.

[0050] The chelate effect is substantial for DNA and RNA molecules because those molecules have repeated phosphate moieties, which, with reference to Fig. 4, the natural pitch of the nucleotide double helix structure often places in close proximity to one another in the major grooves 412 and minor grooves 414 of the DNA tertiary structure. When the phosphate moieties coordinate with cations, the cations reduce the

nucleotide's ability to make conformation changes in the presence of a target analyte. Therefore, such increased rigidity can substantially change the valence or strength of the capture signal produced by an aptamer sensing element when it binds to a target analyte. This effect is especially pronounced in biofluids, such as sweat, that have physiological-range pH levels.

[0051] The size and valence shell of cations have a significant effect on their binding behavior with the DNA structure. Generally, cations with larger atomic radii, and cations carrying increased charge have higher affinity for binding to the phosphate moieties on nucleotides. Recent studies have shown that cation size and valence also influence the exact location of binding within the DNA structure. Specifically, the larger and more highly charged ions tend to bind preferentially within the major groove of DNA. In sweat, the most abundant cations are Na^+ (10-100 mM), H^+ (0.1-0.0001 mM), K^+ (1-24 mM), NH_4^+ (0.5-8 mM), Ca^{2+} (~0.5 mM), and Mg^{2+}. In terms of ionic radius, these abundant sweat cations may be ranked as follows: $K^+ > NH_4^+ > Ca^{2+} > Na^+ > Mg^{2+} > H^+$. Rank according to valence is as follows: $Ca^{2+} > Mg^{2+} > K^+$. Combined, these characteristics produce the following preferential binding orders at the major grooves and the minor grooves of nucleotides in a sweat sample: Major Grooves: $Ca^{2+} > Mg^{2+} > NH_4^+ > K^+ > Na^+$; Minor Grooves: $Na^+ > K^+ > NH_4^+ > Mg^{2+} > Ca^{2+}$. Sweat also contains a number of less abundant ions that will also have preferential binding orders similarly influenced by ionic radius and valence.

[0052] The binding order and location of cations has important implications for the stability of the DNA, and hence DNA behavior as a component of an aptamer sensing element. For example, recent work has shown that the indirect displacement of Na^+ with K^+, Ca^{2+}, or Mg^{2+} in the minor groove of DNA increases the double helix melting temperature, which could in turn affect aptamer conformation or dynamics. Similarly, studies indicate that adding divalent ions that bind specifically to the major groove of DNA improves the reproducibility of analyte-to-aptamer binding for single strand DNA aptamers. For example, in the case of a particular aptamer for detecting glycine, the aptamer binding affinity is directly correlated with divalent ion concentration. In addition to affecting the DNA primary structure, the number of ions bound to an aptamer could also impact the aptamer's dissociation constant or response curve by acting on the aptamer's secondary structure (helical or loop characteristics), or tertiary structure (overlay of loops, helices, etc.).

[0053] Similarly, lanthanide ions (cerium, lanthanum, and gadolinium) have also been identified as enhancing DNA structural stability when reacted with DNA. Kohoutkova, V., *et al.*, "Analysis of DNA modified by cerium (III), lanthanum (III) and gadolinium (III) ions by using of Raman spectroscopy," *J. Biochem Tech* (2010) 2(5):S100-S101. Cerium may be particularly promising for use in biofluid sensing applications due to the strength of its bond with DNA. Because of its strong bond, cerium can remain adhered to the aptamers in EAB sensors for longer periods, promoting long-term stability for such sensors.

[0054] The length of the aptamer sequence treated with small molecules as described herein will influence the effectiveness of such treatments. A nucleotide sequence of less than ten base pairs will tend to maintain a linear secondary structure. However, ten or more base pairs will begin to adopt a helix structure, with a single turn corresponding roughly to each set of ten base pairs. Nucleotide sequences with less than ten base pairs will be stabilized by treatment with small molecules, but to a lesser degree than nucleotide sequences greater than ten base pairs. Longer sequences capable of forming stem-loop or hairpin shapes also tend to show increased structural stability when treated with small molecules than other secondary configurations.

[0055] Therefore, as disclosed herein, one solution to mitigate the EAB sensor performance variabilities caused by fluctuations in biofluid sample salinity is to pretreat the aptamer sections with ions. In particular, aptamer sections may be placed in a saturated ion solution, where the ions are selected to coordinate persistently with nucleotide phosphate moieties (lasting at least for the projected lifespan of the sensing element). Sensor lifespans are expected to range from one hour, to several hours, *e.g.*, 2, 4, 6, 8, or 12 hours, to days, *e.g.*, 1 day, 2 days, 3 days, 5 days, to a week or more. The resulting chelation will yield an aptamer section that is relatively resistant to salinity-induced conformation variability.

[0056] Like the ions discussed above, certain other classes of small molecules also may be used to improve EAB sensor stability. Zwitterionic buffering agents like 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid ("HEPES"), *see* Fig. 5A, and 3-(N-morpholino) propanesulfonic acid ("MOPS"), *see* Fig. 5B, among others, allow for stabilizing interactions with aptamer sensing elements due to their ability to form hydrogen bonds with DNA and RNA molecules. For example, HEPES contains two hydrogens that can serve as electron acceptors, and two carbonyl groups that can be electron donors, while MOPS has one electron acceptor hydrogen and three electron donor groups.

[0057] Hydrogen bonding facilitated by buffering agents can increase aptamer stability by reducing aptamer freedom of movement. First, the mere presence of additional hydrogen bonds between the buffering agent molecules and the aptamer base pairs acts as a type of scaffolding that reduces freedom of movement and causes the aptamer to be more rigid. Additionally, the macro structure of DNA and RNA molecules is highly dependent on hydrogen bonding among the constituent base pairs, which causes the macromolecules to assume various secondary, tertiary, or quaternary folded structures, such as the familiar DNA double helix tertiary structure. The potential added bonds from the buffering agent will therefore create a number of energy states, and will tend to cause the aptamer to fold into a lower energy (and hence preferred) configuration, based on enthalpy and entropy changes. Both the increased structural rigidity, and the assumption of a preferred structural configuration will cause the treated aptamer to display less structural variance in response to salinity variations in the biofluid sample.

[0058] Similarly, treatment with buffering agents will also increase aptamer stability through solvation effects. Like the structural effects of hydrogen bonds, solvation moves the aptamer's macro structure into a more energy-preferred configuration. The biofluid sample, an aqueous solvent, forms solvation shells comprised of concentric solvent layers around the aptamer, as well as around individual buffering molecules. When the buffering molecules coordinate with the aptamer, their respective solvation shells form a composite solvation shell, which changes the configuration and flexibility of the aptamer. These solvation effects force a separation between the biofluid solvent and hydrophobic solute groups on the aptamer and attached buffering agent molecules, which usually manifests by the aptamer folding so that hydrophobic groups are shielded from the solvent. As the aptamer folds around itself to minimize contact between hydrophobic groups and the biofluid, the new structure will have a lower Gibbs energy than it would absent the solvation effects. Therefore, the new configuration will be more stable and less sensitive to changes in biofluid sample salinity variations.

[0059] Other buffering agents that may increase aptamer rigidity include compounds smaller than 1 KDa having at least two hydrogen bond donor/acceptor groups, or at least two Lewis acid or Lewis base groups. These include 1,4-Bis(2-hydroxyethyl)piperazine, *see* Fig. 6, 2-[4-(4-aminobutyl)-1-piperazinyl]ethanol, *see* Fig. 7, and N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid, *see* Fig. 8.

[0060] In other embodiments of the disclosed invention, not only may EAB sensing elements be made less dependent on salinity changes, but other sensing device components that may experience performance variability due to salinity changes can also be pre-treated with an ion or buffering molecule solution to minimize that variability. Studies have shown that ions readily coordinate to organic molecules such as DNA, RNA, cellulose, nitrocellulose, and others based on the ions' charge and size. For example, in cellulose, this coordination takes place between metal ions and the hydroxyl groups located on the glucopyranose backbone. *See, e.g.*, Fig. 9. For example, such ion coordination has been exploited to develop powerful organic solvents capable of promoting the degradation of cellulose networks into individual cellulose chains to enable further chemical processing.

[0061] Similarly, ion and buffering molecule pretreatment has important implications for the use of textile, polymer, membrane, or adhesive materials in wearable biofluid monitoring devices, which may use these materials for attaching, coating, covering, or sealing components, or to comprise components, such as biofluid management components, biofluid concentrator membranes, biofluid collectors, substrates, and wicks or biofluid channels, among others. If such (untreated) materials contact a biofluid sample prior to the sample being measured, the materials could remove enough ions from the sample to introduce concentration measurement errors.

[0062] Correcting for such errors in the context of a biofluid sensing device is a complicated prospect. As a biofluid sample interacts with one of these materials, the material will reach an equilibrium with respect to one or more ions, at which point the material will no longer remove those ions from the sample. The point at which equilibrium is reached will vary for each ion and each material, because each ion will have a different affinity for each material. Further, because sweat ion concentrations in particular can vary over a considerable range, it may be difficult to distinguish between the effects of ion interactions with the materials, and natural concentration variance.

[0063] Therefore, in some embodiments of the disclosed invention, the problem of ion coordination with biofluid sensing device materials may be mitigated by pre-treating the materials in an ion solution to make them relatively inert in relation to biofluid sample ions. The ions in the pretreatment solution would be selected to form persistent bonds, or to coordinate persistently, with the material (lasting at least for the projected lifespan of the sensing device). By doing so, a previously unsuitable or problematic material can

be used in a device and cause less distortion of analyte concentration measurements, and with reduced need to apply corrections to the measured analyte concentrations.

[0064] A material could be pre-treated by incubating it in a solution that contains an ion or buffering molecule with a high affinity for the material's molecular structure. For example, Ca^{2+} ions are a candidate for treating cellulose components in sweat sensing devices because Ca^{2+} ions are relatively dilute in sweat, and have an affinity for cellulose. During the incubation period, the Ca^{2+} ions coordinate to the cellulose backbone of the material and bond there. These ions will remain in place after the treated cellulose is removed from the solution, and will thereby prevent significant ion coordination when in the presence of a biofluid sample. The ion used to pretreat the material will depend on the material, its use in the system, and performance requirements.

[0065] When the treated material is incorporated into a device and encounters biofluid sample ions (*e.g.*, Na^+, K^+, Cl^-) during operation, the relative binding affinities will dictate which ion occupies the binding site. For example, because of the strong affinity of Ca^{2+} ions for cellulose material, the biofluid ions will typically be unable to displace the Ca^{2+} bonded to the material. As a result, Ca^{2+} pretreatment of the cellulose prevents the material from acting as an ion sink during initial operation, or as an ion source during periods of decreased biofluid sample ion concentration. Material ion pretreatment, therefore, can improve system performance by decreasing ion-induced sensor response variability. Other materials would also benefit from pretreatment, such as rayon, polymers, 3D printed polymers, and other materials used for microfluidics.

[0066] This has been a description of the disclosed invention along with a preferred method of practicing the disclosed invention, however the invention itself should only be defined by the appended claims.

ATTORNEY DOCKET NO. ES00746WO

SALINITY-STABILIZED EAB BIOSENSORS

WHAT IS CLAIMED IS:

1. A device, comprising:

one or more sensors for measuring a characteristic of an analyte in a biofluid, wherein each of the one or more sensors includes a plurality of aptamer sensing elements, each aptamer sensing element comprising a nucleotide sequence selected to interact with the analyte; and

a plurality of stabilization molecules configured to chemically coordinate with the nucleotide sequence.

2. The device of claim 1, wherein one or more of the plurality of stabilization molecules is an ion.

3. The device of claim 2, wherein the ion is one or more of the following: a sodium ion, a potassium ion, a calcium ion, a magnesium ion, an ammonium ion, a hydrogen ion, a cerium ion, a lanthanum ion, and a gadolinium ion.

4. The device of claim 1, wherein one or more of the plurality of stabilization molecules is a buffering molecule.

5. The device of claim 4, wherein the buffering molecule is one or more of the following: a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid molecule; a 3-(N-morpholino)propanesulfonic acid molecule; a 1,4-Bis(2-hydroxyethyl)piperazine molecule; a 7, 2-[4-(4-aminobutyl)-1-piperazinyl]ethanol molecule; and a N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid molecule.

6. The device of claim 1, wherein the plurality of stabilization molecules is configured to chemically coordinate with each of the plurality of aptamer sensing elements for a projected lifespan of the device.

7. The device of claim 1, further comprising:

one or more of the following additional components: a wick, a biofluid channel, a substrate, a concentrator membrane, a seal, a coating, and an adhesive; and

a plurality of stabilization molecules configured to chemically coordinate with the additional component.

8. The device of claim 7, wherein one or more of the plurality of stabilization molecules is an ion.

9. The device of claim 8, wherein the ion is one or more of the following: a sodium ion, a potassium ion, a calcium ion, a magnesium ion, an ammonium ion, a hydrogen ion, a cerium ion, a lanthanum ion, and a gadolinium ion.

10. The device of claim 7, wherein one or more of the plurality of stabilization molecules is a buffering molecule.

11. The device of claim 10, wherein the buffering molecule is one or more of the following: a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid molecule; a 3-(N-morpholino)propanesulfonic acid molecule; a 1,4-Bis(2-hydroxyethyl)piperazine molecule; a 7, 2-[4-(4-aminobutyl)-1-piperazinyl]ethanol molecule; and a N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid molecule.

12. The device of claim 7, wherein the plurality of stabilization molecules is configured to chemically coordinate with the one or more additional components for a projected lifespan of the device.

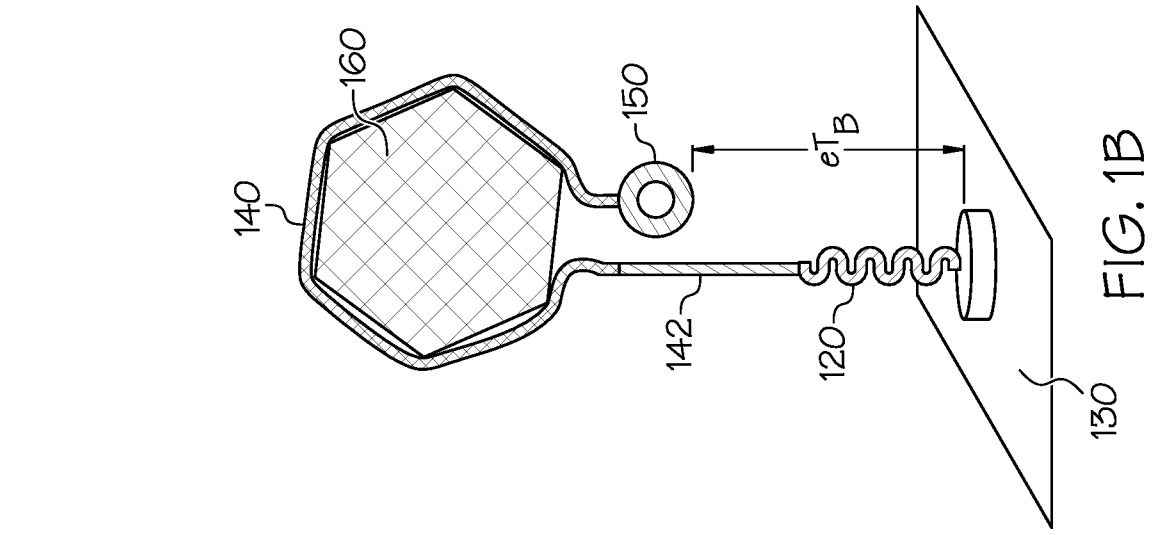


FIG. 1B

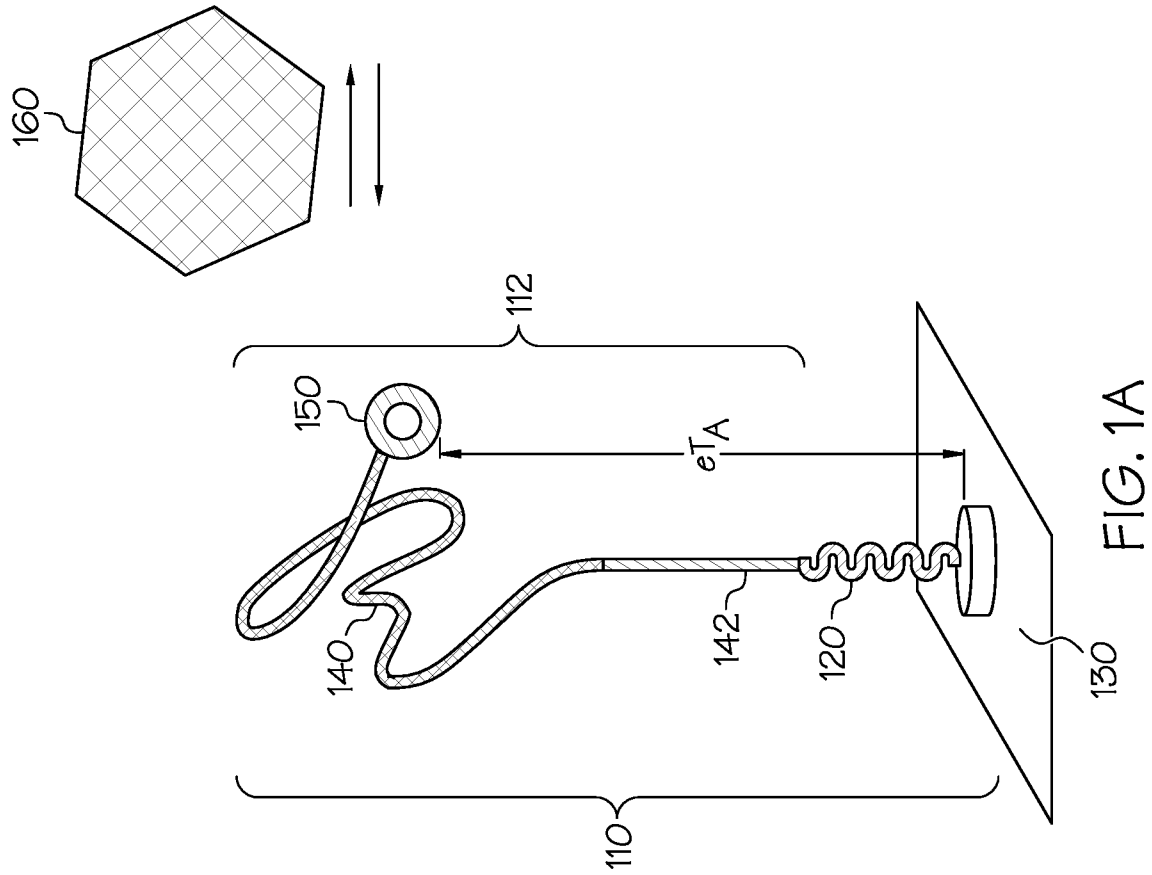
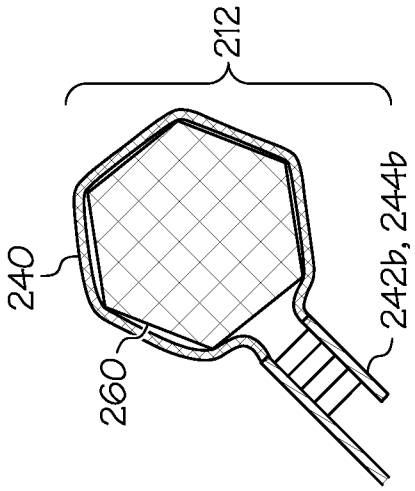


FIG. 1A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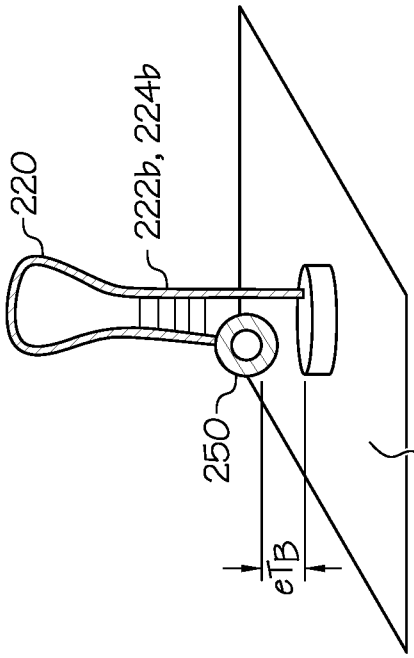
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FIG. 2B

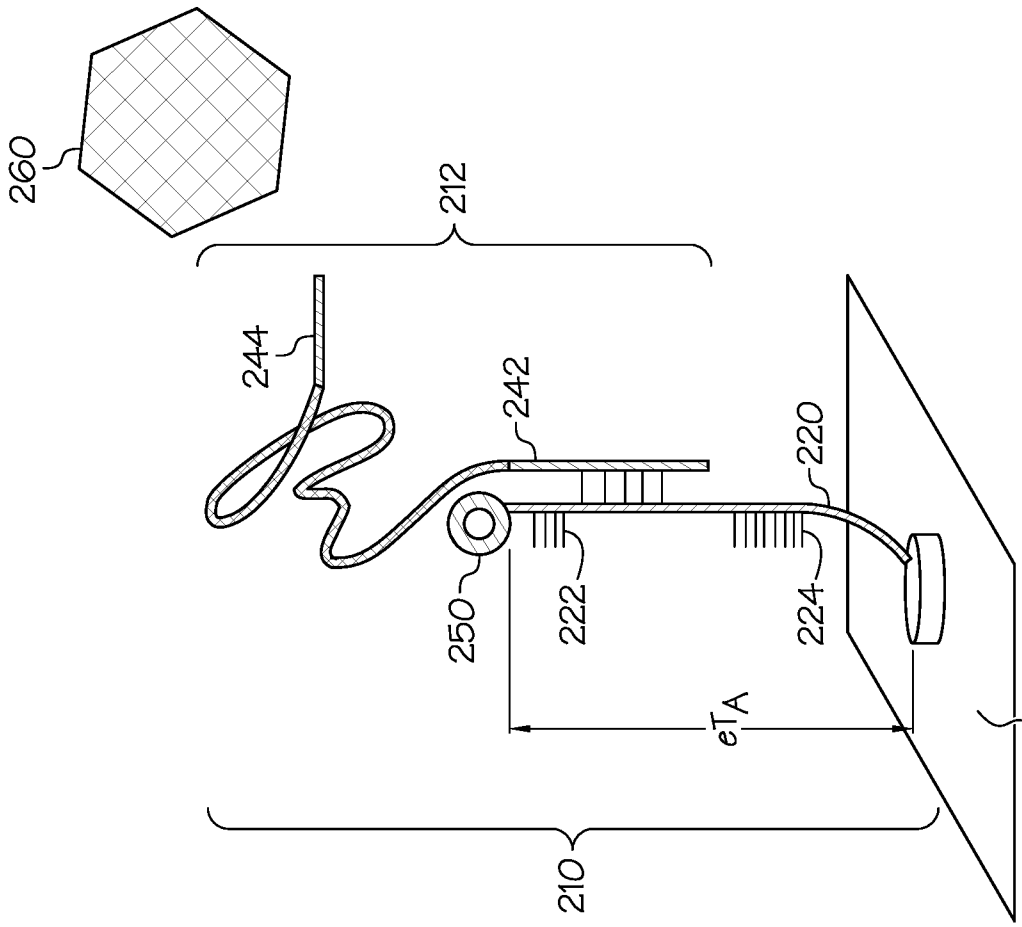
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FIG. 2A

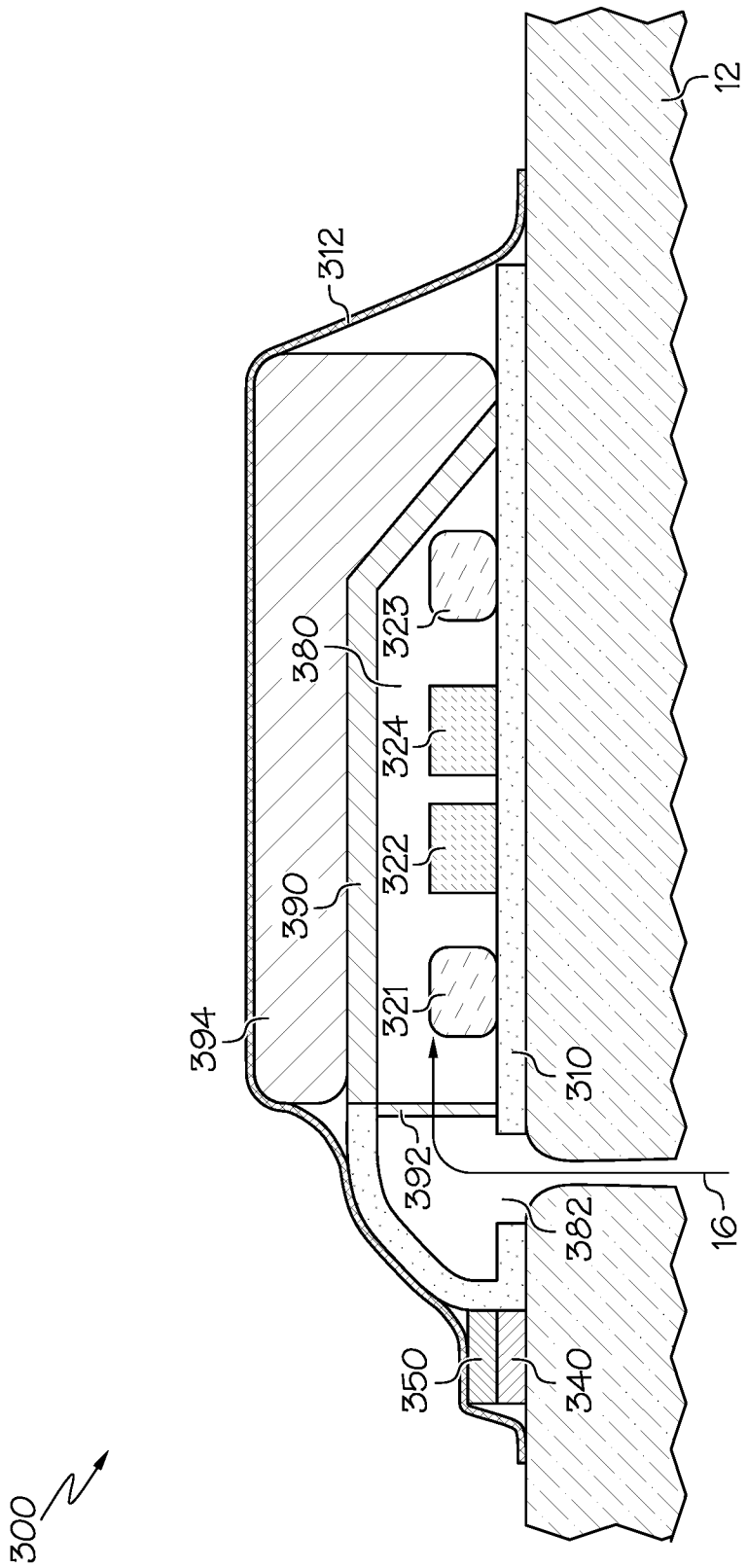

FIG. 3

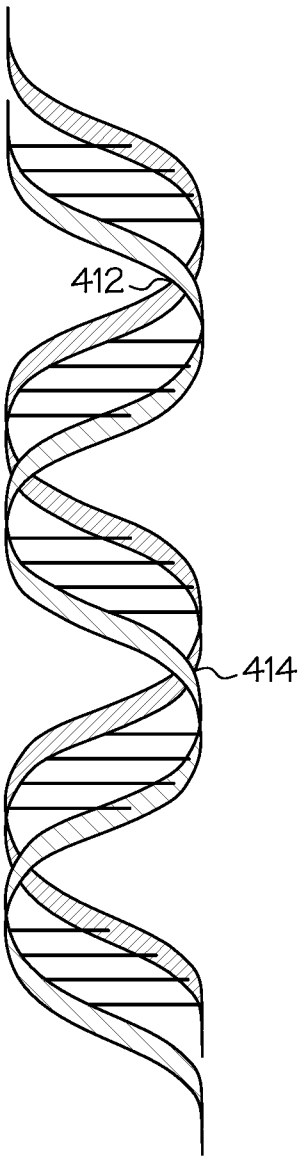

FIG. 4

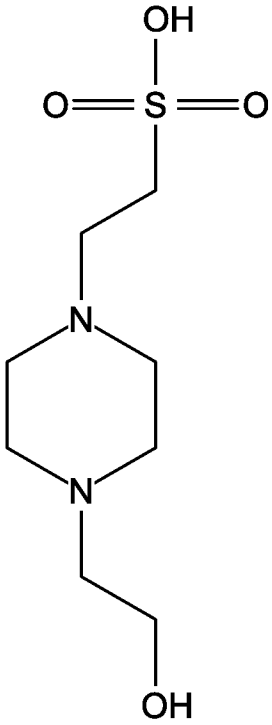

FIG. 5A

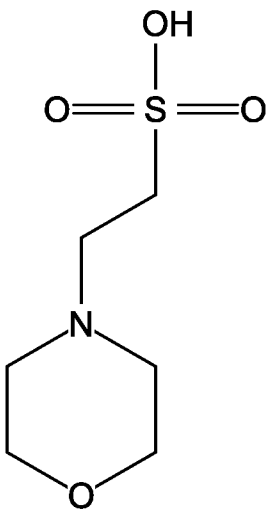

FIG. 5B

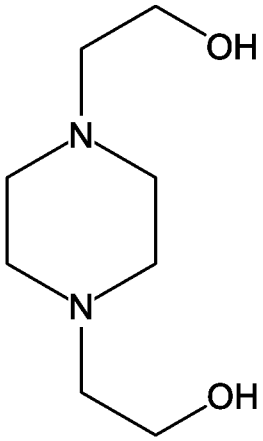

FIG. 6

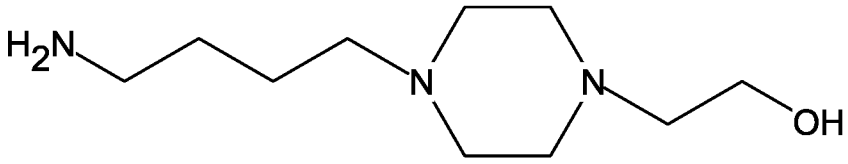

FIG. 7

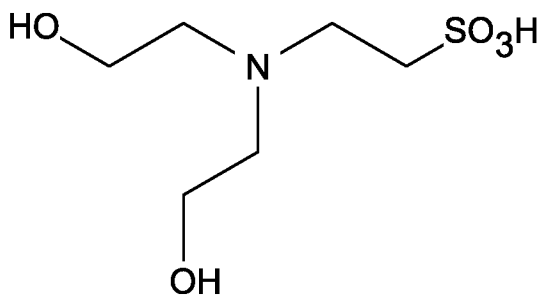

FIG. 8

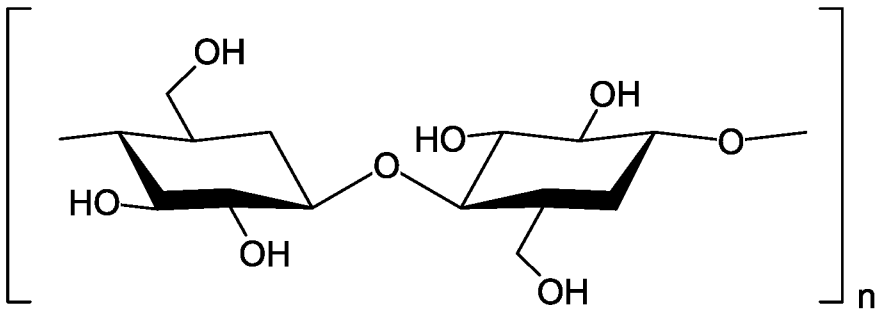

FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/14175

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C23C 16/00, G01N 21/75 (2019.01)
CPC - G01N 33/525, G01N 33/5438

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2017/0245788 A1 (University of Cincinnati) 31 August 2017 (31.08.2017); abstract, para [0008], [0041]-[0042], [0047]	1-12
Y	WO 2016/154034 A1 (VERILY LIFE SCIENCES LLC) 29 September 2016 (29.09.2016); para [05], [082]	1-12
Y	WO 2017/019602 A1 (UNIVERSITY OF CINCINNATI) 02 February 2017 (02.02.2017); para [0020], [0085]	4-5, 10-11
A	WO 2017/070640 A1 (ECCRINE SYSTEMS, INC.) 27 April 2017 (27.04.2017); entire document	1-12
A	US 2014/0335630 A1 (The University of Toledo) 13 November 2014 (13.11.2014); entire document	1-12

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 March 2019	04 APR 2019

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774